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An AMRU-1 strain of *Plasmodium vivax* reverted to chloroquine resistance when selectively passaged in *Aotus* monkeys. A C2A clone of a Mefloquine resistant *Plasmodium falciparum* strain was adapted to splenectomized *Aotus*. Chloroquine resistance reversal was achieved in 2/3 *Aotus* infected with the AMRU-1 strain of *P. vivax* using Chloroquine at 10mg/kg in combination with 20 mg/kg of Prochlorperazine. Oligodeoxynucleotides when given intramuscularly to *Aotus* improve immunogenicity of a *P. falciparum* PADRE 45 peptide immunogen. Intradermal reimunization with an EBA-175, AMA-1, MSP-1 DNA vaccine as a combination with aGM-CSF, partially protected *Aotus* in a rechallenge with a *P. falciparum* FVO strain. Neither, *Aotus* immunized intramuscularlly with EBA-175, AMA-1, MSP-1 DNA vaccine as a combination with aGM-CSF, nor *Aotus* immunized with *P. vivax* Sal-1 blood stage DNA vaccines were protected against an homologous challenge. A significant degree of strain-trascending immunity was observed in *Aotus* that were repeatedily challenged with an FVO strain of *P. falciparum* and then reinfected with an heterologous CAMP strain of *P. falciparum*. *Aotus* that were immunized with an EBA-175, AMA-1 and MSP-1 DNA vaccine intradermally as a combination were not protected when rechallenged with an FVO strain of *P. falciparum*.

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INTRODUCTION

Each year there are 300-500 million new infections and 2-5 million deaths attributable to malaria that occur primarily in countries in the tropics, particularly in sub-Saharan Africa **(4)**. During the past 10-20 years the malaria problem has intensified in some parts of the world because parasites have developed resistance to drugs used for treatment and prevention; the anopheles mosquito, which transmits the parasite to humans, has developed resistance to insecticides, and control efforts have been reduced as resources have diminished in some developing countries **(5)**.

The use of *Aotus lemurinus lemurinus* (Panamanian *Aotus* monkey), cariotypes VIII and IX (11) as a model to study malaria drug resistance and vaccine efficacy, have been ongoing at Gorgas Memorial Laboratory since 1976, due in part to the availability of this monkey in Panama **(15)**, and also to the increasing drug resistance exhibited by the highly pathogenic *Plasmodium falciparum* parasites in Asia, Africa, and Latin America, and more recently *Plasmodium vivax* in the Melanesian and Indonesian archipielago **(16)**. Previously, Schmidt **(21, 22)** used the Colombian *Aotus* as the experimental host for antimalarial drug studies, but embargoes imposed by South American countries on the exportation of monkeys in the mid 1970's seriously restricted the use of *Aotus* for biomedical research in the United States, and in 1976 the project was transferred to Gorgas Memorial Laboratory where Panamanian *Aotus* were available for research. Since then, three strains of *P. falciparum*, Vietnam Smith, Uganda Palo Alto, and Vietnam Oak Knoll, had been adapted to Panamanian *Aotus*. These strains exhibit diverse susceptibility and/or resistance to standard antimalarial agents.

The course of untreated infections in Panamanian *Aotus* has been characterized and compared with that in *Aotus* of Colombia **(20)**. Overall, the virulence of these strains was less in Panamanian than in Colombian owl monkeys, as indicated by lower mortality rates of Panamanian monkeys during the first 30 days of patency. Maximum parasitemias of the Vietnam Smith and Uganda Palo Alto strains were, however, significantly higher during the first 15 days of patency in Panamanian than in Colombian owl monkeys. These quantitative differences in infection parameters between Panamanian and Colombian owl monkeys have not invalidated the use of the former for evaluation of new antimalarial drugs.

Numerous candidate antimalarial drugs of diverse chemical classes have been evaluated against trophozoite-induced infections of one or more *P. falciparum* strains during the course of these contracts. In seeking alternatives to primaquine, two 8-aminoquinolines proved to be active against the blood stages of *P. falciparum* **(2, 13)**. Desferrioxamine, an iron-

specific- chelating agent, was shown to suppress parasitemias of the virulent Uganda Palo Alto strain of *P. falciparum* **(18)**. The *in vitro* activity of two halogenated histidine analogs was not confirmed by evaluation against *P. falciparum* infections in owl monkeys **(17)**.

Chloroquine-resistance of *P. falciparum* represents the greatest challenge in developing effective antimalarial drugs. Reversal of chloroquine-resistance in *P. falciparum, in vitro*, was achieved by the co-administration of verapamil (a calcium channel blocker) plus chloroquine **(12)**. Other in vitro studies have shown that there is a significantly greater efflux of chloroquine from erythrocytes containing falciparum parasites resistant to chloroquine than from red cells parasitized by chloroquine-sensitive falciparum malaria **(9)**. Calcium channel blockers appear to prevent this active efflux of chloroquine, thus allowing the drug to accumulate to parasiticidal levels.

Based upon the success of *in vitro* reversal of chloroquine-resistance, trials were initiated to determine if resistance could be reversed in *Aotus* infected with the chloroquine-resistant Vietnam Smith strain of *P. falciparum*. Six calcium channel blockers, or similarly acting drugs, were co-administered with chloroquine in diverse regimens. The desideratum of chloroquine-resistance reversal was administration of a single course of treatment, with parasite clearance and infection cure. Suppression of parasitemia was obtained during an initial course of treatment, but parasite clearance and cure occurred in some instances only after re-treatment. Such infection parameters were similar to those in monkeys with self-limited infections and cure could be attributed to acquired immunity.

Limited trials with desipramine, Norpramin , a tricyclic psychotropic drug, demonstrated the feasibility of reversing chloroquine-resistance in vivo **(1)**. parasite clearance was obtained, but the infection was not cured.

Subsequently, in vivo reversal of chloroquine resistance was obtained with combinations of chloroquine plus chlorpromazine or prochlorperazine. Such reversal was exhibited by rapid suppression and clearance of parasitemia, resulting in infection cure without retreatment **(10)**.

Evaluation of two oil-soluble derivatives of artemisinin, artemether and arteether, demonstrates that both possess similar activity to cure infections of a multi-drug resistant *P. falciparum* strain in *Aotus* **(23)**.

Some strains of *P. vivax* from Melanesia and the Indonesian archipelago have demonstrated resistance to treatment with chloroquine **(14, 19)**. Unlike chloroquine-resistant falciparum malaria, there exists no easy alternative to chloroquine-resistant strains of vivax malaria. Using WR 238605 alone or in combination with chloroquine in Panamanian *Aotus* monkeys it was demonstrated that WR238605 is a an alternative treatment for chloroquine-resistant vivax malaria **(16)**. The compound WR 238605 is a

primaquine analog developed by the US Army as a better tolerated, more effective replacement for primaquine.

Both the purpose and methods of approach of the present work remains essentially unchanged since 1976, viz to ascertain the antimalarial activity of drugs against *P. falciparum* and *P. vivax* in *Aotus*. The method of approach may vary on an ad hoc basis, such as administering a combination of drugs.

The long term goal of the second part of this project is to develop fully protective plasmid DNA vaccines that induce protective immune responses against the sporozoite, liver and erythrocytic stages of *P. falciparum*. If successful, it will establish, for the first time, that plasmid DNA vaccines can protect non-human primates, a critical step forward for the use of plasmid DNA vaccines in humans.

Vaccines are aimed at inducing immune responses that disrupt the complex cycle of the parasite at one more points: anti-sporozoite antibodies that prevent invasion of hepatocytes; cytotoxic T lymphocytes, cytokines, and antibodies that eliminate infected hepatocytes; antimerozoite antibodies that prevent invasion of erythrocytes; antibodies that neutralize parasite exoantigens known to induce harmful cytokine responses; antibodies that attack infected erythrocytes; cytokines that kill parasites within erythrocytes; and, anti-sexual stage antibodies that prevent the development of sporozoites in the mosquito.

Previous trials of malaria blood stage vaccines have shown that the Panamanian *Aotus*\|*P. falciparum* model to be suitable for this purpose. **(6-8)**.

Immunogenicity studies of a plasmid DNA vaccines encoding the circumsporozite *P. yoelli* rodent malaria gene (PyCSP) in Panamanian *Aotus* monkeys demonstrated that the intradermal route of inoculation (ID) induces a higher level of antibodies than the intramuscular route (IM). Antibody levels induced in this manner reached a peak at week 9 and titers declined to 50% their peak value by week 14. When boosted at week 46 antibody levels increase 4 fold by week 49. This was comparable to antibodies generated with a Multiple Antigen synthetic peptide vaccine (MAP) delivered with an adjuvant **(4)**

We have used this inmunization scheduled to test single or multi-gene DNA plasmid vaccines in *Aotus* monkeys. Additionally we have tested the ability of recombinant cytokines to enhance the immunogenicity and protective efficacy of the DNA vaccines. Preliminary studies (previously described in the 1996 Annual Report) using a small group of *Aotus l. lemurinus* (n = 3) demonstrated partial, but incomplete, protection with a DNA vaccines for either AMA-1 or EBA-175 alone. These studies indicated that animals which received the vaccine candidates, had a short, but

apparent significant delay in the onset of parasitemia {approximately 33% (1 of 3) self-cured, whereas none of the control animals did}. However, since the number of animals per group in each of these pilot studies were small, it was not possible to determined the absolute efficacy of these candidate vaccines, but these experiments suggested to the investigators that further studies were warranted. MSP-1, when used as a protein/peptide vaccine formulation, provided protection from a *P. faciparum* infection in *Aotus* monkeys and we have demonstrated that, in mice and in Rhesus monkeys, the cytokine GM-CSF augmented both immunogenicity of a malaria DNA vaccine (personal communication. W. Weiss). We have now completed a pilot experiment to determine if *Aotus* Granulocyte-Macrofage-Colony Stimulating Factor (aGM-CSF) can augment immunogenicity and protective efficacy of a multi-gene erythrocytic vaccine.

We have also tested the effect of prior *P. falciparum* infection on the immunogenicity of a DNA vaccine, obtaining partial protection in 67% of the monkeys. (See previous annual report).

The purpose of this report is to: 1) Present data on the evaluation of potential antimalarial activity of drugs in the pre-clinical model of *Aotus l. lemurinus* (Panamanian night monkey) experimentally infected with *P. falciparum* or *P. vivax*, and 2) data on plasmid DNA malaria vaccine experiments. These studies were supported by the U.S. Army and the U.S. Navy Malaria Programs.

BODY

I. Experimental Methods

The first aim of this project is to evaluate the potential antimalarial activity of drugs, or combination thereof, in the preclinical model of *Aotus* experimentally infected with *P. falciparum* (or *P. vivax*). Specifically, the vertebrate host is *A. l. lemurinus*, the Panamanian night monkey. These animals are either feral, laboratory adapted or laboratory born. No naturally acquired, human plasmodium infection has been reported in *Aotus*. The Vietnam Smith/RE strain of *P. falciparum* was adapted to *Aotus* of Colombian origin in 1971 **(21)** and in Panamanian *Aotus* in 1976. **(20)**. The course of untreated infections, essential for comparison with treated infections, has been documented in Panamanian *Aotus* **(20)**. This plasmodium strain is resistant to maximally tolerated doses of chloroquine, pyrimethamine, and quinine **(22)**.

To initiate an experiment, infected blood (with 2.5% sodium citrate as the anticoagulant) from an untreated *Aotus* was diluted appropriately in chilled saline (0.85%), such that each milliliter contained 5,000,000 parasites. This amount was inoculated into the saphenous vein of experimental and control monkeys.

Blood films, prepared and examined daily beginning on the first post-inoculation day, were stained with Giemsa. Parasitemias were evaluated as follows: negative, if no parasites were detected on a thick blood film after examination for at least 5 minutes; <10 parasites per cmm, if positive only on the thick blood film; parasite enumeration was by the Earle-Perez method and reported as the number of parasites per cmm. **(3)**

Blood films from untreated *Aotus*, serving as passage and/or control subjects, were prepared and examined daily during the primary patent period, and daily thereafter for at least three consecutive days after parasites could last be detected on thick blood films. When parasitemia had cleared, films were made and examined twice weekly until a total of 100 negative days had been recorded. If recrudescence occurred, blood films were obtained again on a daily basis.

Parasitemias were evaluated daily during the treatment period and until blood films were negative for at least seven consecutive days. The frequency of smearing was then reduced to two times per week (Monday and Thursdays or Tuesdays and Fridays). If no recrudescences occurred during a 100 day examination period, the infection was considered to have been cured.

Drug doses were calculated as mg base per kg of body weight. Stock solutions of water soluble compounds, at appropriate concentrations, were

prepared with distilled water and stored at 8° C for the treatment period. If a compound was water insoluble, a suspension of the requisite amount of drug was prepared daily with 0.3% methylcellulose (in distilled water).

Oral administration of drugs was by gastric intubation with a 14 French catheter. The total volume of fluid administered, drug solution or suspension, and rinse was 14 ml.

Response to treatment was categorized as clearance and cure, clearance and recrudescence, or suppression withouth clearance. The day of clearance was defined as the first of three consecutive days in which the thick blood films were parasite negative. The day of recrudescence was the first of three consecutive days of positive thick blood films after a period of clearance. Suppression was defined as a transient decrease in the parasite count post-treatment without clearance.

The second objective of this project is to evaluate plasmid DNA vaccines against the blood and sporozoite stages of *P. falciparum* and against the blood stages of *P. vivax* in the Panamanian *Aotus* model. To this end we have evaluated single and multigene DNA vaccines of *both P. falciparum* and *P. vivax* with or without the addition of cytokines. The results of these experiments are detailed in results.

II. Results

A. Passage of a Chloroquine resistant AMRU-1 strain of *Plasmodium vivax* in *Aotus* monkeys.

On 29 October 1998, one *P. falciparum* cured *Aotus* was inoculated intraperitoneally (IP) with a frozen AMRU-1 strain of *P. vivax*. This animal was followed up with daily blood smears for evidence of parasitemia until it reached 4,870 parasites x *ul* on day 20 Post inoculation (PI) and then treated with 10 mg/kg of Chloroquine for five days. One ml of infected blood from this animal with less than 10 parasites x *ul* was collected and passaged into another *Aotus* on 4 December 1998, when its parasitemia reached 25,670 parasites x *ul* was treated with 10 mg/kg of Chloroquine for three days. Blood from this animal was freeze on day 19 PI when its parasitemia was 37,090 parasites x *ul*. Parasitemia remained high despite treatment and the animal self cured on day 36 PI. A third animal inoculated sequentially on 21 January, 1999 with frozen stock IP was positive on day 5 PI. This animal was used as donor for a drug evaluation study.

B. Adaptation of Mefloquine resistant *P. falciparum* strains WR75 and clones C2A and C2B to *Aotus* monkeys.

Mefloquine resistant strains of *P. falciparum* have been detected along the Cambodia-Thailand border in Asia. These strains have been studied *in vitro* but until now adaptation to *Aotus* has been unsuccessful. The purpose of this experiment was to adapt a Mefloquine resistant WR75 *P. falciparum* strain and its clones C2A and C2B to *Aotus* monkeys in order to do future drug resistant studies *in vivo*. On December 14, 1998 three splenectomized *Aotus* were inoculated Intravenously (IV) and IP with 1 and 3 mls respectevily of cultured *P. falciparum* parasites strains WR75 and clones C2A and C2B brought from WRAIR. Seventy three days after inoculation the C2A inoculated monkey became positive with a peak parasitemia on day 84 of 10,500 parasites x *ul.* Blood from this monkey was passage into another splenectomized one, this time becoming positive on day 2 PI. Blood was cryopreserved when reached 7,550 parasites x *ul.*

C. Reversal of chloroquine resistance with the co-administration of prochlorperazine (WR280001AC; BN 43106) and chloroquine (WR1544 BM;AR 20613) against infections of the AMRU-1 strain (CQR) of *Plasmodium vivax*.

Previous studies with a CQR *P. falciparum* have shown that it is possible to achieve *in vivo* reversal of CQR by the co-administration of prochlorperazine and chloroquine, as evidenced by infection cure. Neither drug alone effects such cure. In one study with the CQR AMRU-1 strain of *P. vivax*, data indicated that WR238605 (a primaquine analogue) administered at 1.0 mg/kg x 3, plus chloroquine (10.0 mg/kg x 3) cured 2 of 3 infections, WR238605, alone at this dose, clears parasitemia but with recrudescence. The present study is designed to determine if CQR of the AMRU-1 strain can be reversed *in vivo* by prochlorperazine plus chloroquine. On 21 January, 1999 a donor *Aotus* monkey was inoculated with frozen stock of the AMRU-1 strain of *P. vivax*. Each of 7 *Aotus l. lemurinus*, cured of *P. falciparum,* males and females, were inoculated on 3 February, 1999 intravenously with 5 x 10^6 of *P. vivax* AMRU-1 strain parasites. Blood films were obtained on the day after inoculation and continued daily for the duration of the experiment. When parasitemias approximates 5,000 per cmm, oral treatment for three days was initiated as follows: Group 1. Three monkeys received Prochlorpeazine 20 mg/kg plus chloroquine 10 mg/kg x five days. Group 2. Three monkeys received Chloroquine 10.00 mg/kg x five days. Group 3. Untreated control. Infections were considered cured, when films remained negative for 100 days. Recrudescense will be

treated on an ad hoc basis. As shown on Table 1, 2/3 monkeys from group 1 cleared parsitemias on the first day after treatment and remained negative for more than 16 days post-inoculation (PI), the day of this report. In group 2 and 3 all animals remained positive for more than 16 days PI.

D. Augmentation of PADRE 45 immunogenicity with CpG in *Aotus* monkeys.

This experiment was started in 05 May 98 in order to determine the relative immunogenicity of a synthetic peptide derived from the PfCSP sequence (PADRE 45) with different CpG sequences, emulsified in Montanide and delivered IM to *Aotus* monkeys.

The rationale for this experiment was that CpG sequences (short synthetic DNA sequences modeled from bacterial DNA) will enhance the immunogenicity of PADRE 45 when delivered IM emulsified in Montanide ISA720 in *Aotus* monkeys.

Three groups of 3 animals each were injected unilaterally in the quadriceps (400 µl total volume). A total of 100 µg of PADRE 45 and 500 µg of one of three CpG sequences were injected per dose as follows: Group 1:PADRE 45 in Montanide 720 plus ODN 1968; Group 2: PADRE 45 in Montanide 720 plus ODN 2041; Group 3:PADRE 45 in Montanide 720 plus ODN 2006.

All animals were bled several times before and after immunization at two week intervals on 05 May, 25 May, 4 June, 15 June, 30 June, 14 July, 27 July, 11 August and 8 September and immunized three times, 05 May, 26 May and 16 June 1998. No challenge was carried out in this experiment. The animals receiving oligodeoxynucleotide containing either three of four CpG motifs produced antibodies that bound a recombinant CSP as measured in ELISA, and reacted with *P. falciparum* sporozoites as tested in a sporozoite immunofluorescent test. These responses were significantly greater than those seen in animals receiving the oligodeoxynucleotide withouth CpG motifs. These data indicate that oligodeoxynucleotides containing CpG motifs improve immunogenicity of peptide immunogens in non-human primates an may be immunopotentiators useful in humans. A manuscript that reports these results has been accepted for publication by *Vaccine*.

E. Immunogenicity and Efficacy of a *P. falciparum* EBA-175, AMA-1, MSP-1 DNA Vaccine as a combination delivered intradermally with or without *Aotus* Granulocyte-Macrophage-Colony-Stimulating Factor (aGM-CSF) in *Aotus* Monkeys.

As shown on the previous report, twelve malaria naive Aotus immunized intradermally with a combination erythrocytic stage malaria plasmid DNA vaccine, consisting of EBA-175, MSP-1 and AMA-1 with or without co-delivery of an expression plasmid encoding an *Aotus* aGM-CSF, were not protected when challenged with 1 x 10^5 parasites of a *P. falciparum* FVO on January 19, 1998. Nine of the 12 originally recruited animals for this experiment were re-immunized on 1 December, 1998 and then re-challenged on 11 January, 1999 with 10,000 parasites of the FVO strain of *P. falciparum*. Sera were collected every two weeks beginning the day prior to the FVO infection and continuing every two weeks after infection. As shown on table 3 seven days after challenge a naive control became positive and was treated on day 12 PI when parasitemia reached 247,640 parasites x *ul*. One animal from group 2, another one from 4 and a re-challenge control animal became positive on day 10 PI, the rest except for two other animals became positive between days 12 and 14 PI. One animal from group 1 remained negative for more than 25 days. One animal from group 3 had a peak parasitemia of 1,210 parasites x *ul* and then self cured on day 23 PI. Another one from group 4 had a peak parasitemia of 1,040 parasites x ul self curing on day 18 PI. The rest had to be treated with mefloquine as follows: One animal from group 1 on day 20 PI due to a low hto reading. Two animals from group 2 on day 20 PI when they went over the 300,000 parasites threshold. One of these animals died malaria-associated causes despite being treated with mefloquine at 390,000 parasites/*ul*. One animal from group 3 was treated on day 21 and another one from group 4 on day 22 due to a low Hto reading. In conclusion only half of the animals from group 1 were protected from challenge in this experiment.

F. Immunogenicity and efficacy of a *P. falciparum* EBA-175 , AMA-1, MSP-1 DNA vaccine as a combination with or without aGM-CSF in *Aotus* monkeys immunized by the intramuscular route.

Aotus granulocyte-monocyte colony stimulating factor (aGM-CSF) is a cytokine that drives hemopoeitic stem cells to produce more cells of granulocytic and monocytic lineage. Previous studies have demonstrated a lack of immunogenicity of a DNA vaccine administered IM in *Aotus* monkeys. GM-CSF was incorporated into this multi-gene DNA vaccine

protocol and administered IM to determine if GM-CSF can reverse the failure of the DNA vaccines alone to induce an effective immune response.

The objectives of this experiment was to compare the immunogenicity and protective efficacy of a combination erythrocytic stage malaria vaccine consisting of EBA-175, MSP-1, and AMA-1 with and without co-delivery of an expression plasmid encoding aGM-CSF when injected by the IM route.

The experiment consisted of two groups of six monkeys each which received: Group 1. AMA-1, EBA-175 and MSP-1 DNA vaccines IM and the 1012 vector without insert. Group 2 received plasmid backbones without insert plus aGM-CSF. Three naive animals served as non-vaccinated controls.

All animals were bled several times before and after immunization at two week intervals and immunized four times, 8 April, 01 June, 29 June and 1 September 1998. Challenge was carried out on 9 October, 1998 with 10,000 parasites IV of an FVO strain of *P. falciparum*.

As shown on table 4 all animals became patent by day 7 PI. Treatment with 40 mg/kg of mefloquine once, was initiated on day 11 PI in one animal from group 2 when it reached 400,000 parasites x *ul*. On day 12 PI, three animals from group 1 and three from group 2 including two naive controls had to be treated. On day 13 PI another naive control was treated. By day 18 PI one animal from group 2 was treated this time due to a low hto reading. Only one animal from group 1 selfcured on day 19 but recrudesce on day 42 PI (20 November, 1998) with a peak parasitemia on day 49 PI of 110,250 parasites x *ul* being treated on day 56 PI (December, 4 1998) due to a low hto reading. Serologicals results are pending. Two animals, one from group 1 and another one from group 2, died of unrelated causes before challenge. In conclusion no significant difference was observed between groups in this experiment.

G. Immunogenicity and Efficacy of *P. vivax* DNA vaccines based on PvCSP, PvSSP2, PvMSP-1p42, PvAMA1, and PvDBP(regions II-IV) alone or in combination in *Aotus* Monkeys.

As previously reported on the 1998 annual report, this experiment was started on 29 October 97 in order to evaluate the immunogenicity of five components of a multi-component DNA vaccine against *P. vivax*, PvCSP, PvSSP2, PvMSP1(p42), PvMA1, PvDBP (regions II-IV) and to test the efficacy of the multi-component vaccine against a blood stage challenge. The experiment consisted of seven groups of monkeys. The first four groups (3 animals each) were immunized with a PvCSP (Group 1), PvSSP2 (Group 2), MSP-1(p42) (Group 3), AMA-1 (Group 4). The primary purpose of these four groups was to test immunogenicity of these four

individual components. The final three groups included 8 monkeys each that were immunized with PvDBP (regions II-IV) (Group 5), a mixture of the five individual plasmids (Group 6), and a negative control plasmid (Group 7). These groups were evaluated for vaccine immunogenicity. Each monkey received 500ug/plasmid/dose, given intradermally at weeks 0, 4, 8, and 20. Challenge occurred on 27 April with 1 x 10^6 parasites of a *P. vivax* Sal-1 strain. Thirty-five animals and two *P. vivax* naive controls were inoculated. One animal from group five died before inoculation due to unrelated causes. As shown on table 5, no significant differences were found between groups in regard to prepatent period, days to peak parasitemia, or self-cured rates.

The prechallenged IFA titers against sporozoites (spz) or infected erythrocytes (irbc) were as follows Group 1 (PvCSP) 1:5120 spz; Group 2 (PvSSP2) 1:320 spz; Group 3 (PvMSP1) 1:2560 irbc; Group 4 (PvAMA1) 1:1280 irbc; Group 5 (PvDBP) <1:10 irbc; Group 6 (5 gene mixture) 1:5120 spz, 1:320 irbc; Group 7 (negative control plasmid) <1:10 spz, <1:10 irbc. Following challenge there was a suggestion that the parasitemias in the monkeys immunized with PvMSP1 were lower than in other groups, however, this was not statistically significant in this experiment. The irbc IFAT titers following challenge were very high in all groups, suggesting that they may have been primed by cross reacting antigens from their previous exposure to *P. falciparum*.

H. Heterologous *Plasmodium falciparum* CAMP strain blood stage challenge of hyperimmune *Aotus* monkeys.

The objective of this experiment was to determine whether repeated challenge with one strain of *P. falciparum* induces immunity in *Aotus l. lemurinus* to blood stage challenge with a heterologous strain of *P. falciparum*, the CAMP strain. On 21 September 1998, eight *Aotus* monkeys that had already undergone seven previous *P. falciparum* FVO infections were challenged with 10,000 parasites of the CAMP strain, a strain of parasite originally isolated in Malaysia. Although FVO was isolated from Vietnam, genetic analysis shows that the two strains have a variety of allelic differences in the sequences of antigens of interest to vaccine developers. All animals were previously treated on 7 September with 50 mg quinine once a day for 5 days and 10 mg of Doxycycline once to eliminate any sub-patent FVO strain infections. Daily blood smears for parasite counting and blood dots on filter paper were taken for detection of any sub-patent FVO or CAMP infections using PCR directed against specific sequences in the genes encoding blood stage antigens. Sera were collected every two weeks beginning the day prior to the CAMP infection and continuing every two weeks after infection. Three *P. falciparum* naive

controls were used. As shown on table 6 all became parasitemic by days 6 and 7 PI. Five hyperimmunized animals became parasitemic between days 7-9 PI. One became parasitemic on day 14 PI and the other two did no show evidence of parasites in their blood for more than 40 days PI. Control naive animals were treated with mefloquine 40 mg/kg on days 12 and 13 PI when they reached 400,000 parasites x ul. Parasitemias in the hyperimmune group ranged between <10 and 10,000 parasites x *ul* selfcuring between days 16-18 PI. No recrudescences were observed for 112 days PI. This experiment concluded on 1/11/99 when the animals were considered cured. During this experiment it was observed that the prepatency period increases and the severity of infection decreases with each successive infection. After five infections 50% of the animals were immune; after six infections, all were immune. Subsequent challenges with blood stage parasites of a heterologous strain (CAMP) either failed to become parasitemic (2/8) or self-cured their infections (6/8). These findings indicate that a significant degree of strain-transcending immunity developed during the repetitive challenges with FVO, in spite of the measurable heterogeneity in the sequences of several parasite proteins of interest to malaria vaccine developers. A manuscript has been completed and will be submitted for publication shortly.

I. Immunogenicity and efficacy of a *P. falciparum* EBA-175, AMA-1 and MSP-1 DNA vaccine alone or in combination in *Aotus* Monkeys.

As shown on the previous annual report, Aotus immunized with AMA-1, EBA-175 and MSP-1 as a combination were not protected against a challenge with a *P. falciparum* FVO strain, all animals in Groups 4 and 5 became parasitemic with no detectable differences in prepatent period, days to peak parasitemia or day of intitiation of treatment. When these animals were re-challenged on 28 July 1998 with 10,000 parasites of a *P. falciparum* FVO strain. As shown on Table 7 all animals became parasitemic, this time between day 10 and 11 PI. One naive control animal became parasitemic on day 6 PI and the other one on day 11 PI. One of these animals was treated on day 14 PI with mefloquine 40 mg/kg once. On day 16 PI one animal from group 4 was treatment with 10 mg/kg of Quinine for five days.

Its parasitemia was suppressed for two days but went up to 533,990 parasites x ul on day 19 PI when it was decided to treat it with 40 mg/kg of mefloquine once due to an apparent resistance to quinine of the FVO strain. Quinine treatment was initiated in four animals from group 4 and two from group 5 on day 19 PI, but then were retreated with 40 mg/kg of mefloquine on day 20 PI because the animal that was first treated with quinine on day 16 PI died of malaria. Two other animals, one from group 4 and another one from group 5, were treated with mefloquine on day 21 PI. On day 22 PI five animals, two from group 4 and two from group 5 were treated. Of these, one animal from group 5 died despite treatment and another one that was treated the day previously died also. The second naive control was then treated with mefloquine due to a low hto reading. No significant differences were found between groups in regard to prepatent period, days to peak parasitemia, or day of treatment.

CONCLUSIONS

The AMRU-1 strain of *P. vivax* reverted to chloroquine resistance (CQR) when selectively passaged and treated with chloroquine at 10 mg/kg for five days in *Aotus* monkeys.

A C2A clone of a Mefloquine resistant *P. falciparum* strain was adapted to splenectomized *Aotus* after a 74 day prepatent period.

Chloroquine resistance reversal was achieved in 2/3 *Aotus* infected with the AMRU-1 strain of *P. vivax* by using chloroquine at 10mg/kg and prochlorperazine at 20 mg/kg in combination.

Oligodeoxynucleotides (CpGs) when given intramuscularly to *Aotus* improved immunogenicity of a *P. falciparum* PADRE 45 peptide immunogen.

Reimmunization and rechallenge with a *P. falciparum FVO* strain partially protected 1/2 *Aotus* that received an EBA-175, AMA-1, MSP-1 DNA vaccine as a combination with aGM-CSF intradermally.

Aotus immunized intramuscularlly with EBA-175, AMA-1, MSP-1 DNA vaccine as a combination with aGM-CSF were not protected against *a P. falciparum* FVO challenge.

Aotus immunized with *P. vivax* blood stage DNA vaccines were not protected against a *P. vivax* Sal-1 challenge.

A significant degree of strain-transcending immunity developed in *Aotus* that were challenged repeatedily with an FVO strain of *P. falciparum* and then infected with a heterologous CAMP strain of *P. falciparum*.

Aotus that were immunized with an EBA-175, AMA-1 and MSP-1 DNA vaccine intradermally as a combination were not protected when rechallenged with an FVO strain of *P. falciparum*.

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TABLE 1

DETAILED ACTIVITY OF PROCHLORPERAZINE* (WR280001AC;BN43106) AND CHLOROQUINE** (WR 1544BM;AR20613) AGAINST INFECTIONS OF THE AMRU-1 STRAIN (CQR) OF *Plasmodium vivax* in Aotus monkeys.

MONKEY #	DAY P.I.	RX INITIATED DAY PAT.	MG/KG	PARASITEMIA PER CMM X 103 DAY PRE. RX	DAY OF RX 1	2	3	4	5	DAY POST RX 1	2	3	4	DAYS NEG.
12894	5	1	20* 10**	1.9	7.5	21.7	4.5	0.01	0.01	0	0	0	0	16
12900	5	1	20* 10**	1.7	10.5	26.2	2	0.01	0.01	0	0	0	0	16
12940	5	1	20* 10**	1.5	9	15.7	33.2	46.8	34.7	12	7.5	6	2.9	
12914	5	1	10**	0.76	4.09	8.4	12	22.5	7.09	6	1.6	4.5	1.3	
12911	5	1	10**	0.01	2.9	0.65	19.6	7.5	22.65	39.2	9	28.6	1.5	
12906	5	1	10**	1.04	6	36.7	15.1	14.8	6.04	2.9	1.7	3.9	8	
12910	5	1	CONTROL	1.06	5.7	19.5	45.1	48.3	48.5	24.1	25.8	24.1	24.1	
12943	5	1	CONTROL	0.47	4.9	17.4	16.5	66.4	60.4	60.4	49.8	40.7	43.7	

TABLE 2

SUMMARY OF ACTIVITY OF PROCHLORPERAZINE* (WR280001AC;BN43106) AND CHLOROQUINE**
(WR 1544BM,AR20613) AGAINST INFECTIONS OF THE AMRU-1 STRAIN (CQR) OF *Plasmodium vivax* in Aotus monkeys

MONKEY #	Daily Dose x 5 days Mg/Kg	Response of parasitemia to Rx			Days from final Rx to parasite clearance	Days from final Rx to recrudescence	Notes No. of days negative
		None	Suppressed	Cleared			
12894	20*			X	1		16
	10**						
12900	20*			X	1		16
	10**						
12940	20*	X					
	10**						
12914	10**	X					
12911	10**	X					
12906	10**	X					
12910	CONTROL	X					
12943	CONTROL	X					

TABLE 3

DETAILED PARASITEMIA OF AOTUS VACCINATED WITH A PLASMID DNA VACCINE EBA-175, AMA-1, MSP-1
INTRADERMALLY AS A COMBINATION WITH OR WITHOUT aGM-CSF AND RECHALLENGE WITH A *P. falciparum* FVO STRAIN

Parasites x cmm

MONKEY DAY/PI	GROUP	DAY/PI 1	2	3	4	6	7	8	9	10	11	12	13
12876	1	0	0	0	0	0	0	0	0	0	0	0	<10
12882	1	0	0	0	0	0	0	0	0	0	0	0	0
12884	2	0	0	0	0	0	0	0	0	<10	0	<10	<10
12885	2	0	0	0	0	0	0	0	0	0	0	<10	<10
12888	3	0	0	0	0	0	0	0	0	0	0	0	0
12890	3	0	0	0	0	0	0	0	0	0	0	0	<10
12889	4	0	0	0	0	0	0	0	0	0	0	0	0
12891	4	0	0	0	0	0	0	0	0	0	0	0	0
12892	4	0	0	0	0	0	0	0	0	<10	0	<10	<10
12901	CONTROL	0	0	0	0	0	0	0	0	<10	<10	>10	1040
12935	NAIVE	0	0	0	0	0	<10	>10	310	45300	51090	247640*	

MONKEY DAY/PI	GROUP	14	15	16	17	18	19	20	21	22	23	24	25	26
12876	1	>10	26250	16620	37750	38010	11570	4530*						
12882	1	0	0	0	0	0	0	0	0	0	0	0	0	
12884	2	>10	8250	10570	107640	223480	202340	413090*						
12885	2	>10	80300	64930	163080	167610	289920	390990*	158690	DIED				
12888	3	0	<10	<10	>10	>10	410	1210	610	<10	<10	<10	<10	
12890	3	<10	5750	31710	110990	25670	178180	102680	138920*					
12889	4	<10	6290	9060	33220	48320	71090	83050	90600	67950*				
12891	4	0	<10	<10	1280	9060	8110	27180	16610	10570	3300	<10	<10*	
12892	4	<10	<10	0	<10	0	0	0	0	0	0	0	0	
12901	CONTROL	18010	94000	77010	271800	295390	407360*							
12935	NAIVE													

*treatment

TABLE 4

DETAILED PARASITEMIA OF AOTUS VACCINATED WITH P. falciparum EBA-175, AMA-1, MSP-1
DNA VACCINES AS A COMBINATION WITH OR WITHOUT aGM-CSF BY THE INTRAMUSCULAR ROUTE.

Parasites x cmm

MONKEY	GROUP	DAY/PI 1	2	3	4	5	6	7	8	9	10	11
12921	2	0	0	0	0	0	0	<10	1180	30910	78540	133200
12920	1	0	0	0	0	0	0	<10	660	18490	63140	109340
12923	1	0	0	0	0	0	0	<10	510	21560	92400	99760
12922	2	0	0	0	0	0	0	<10	230	30800	83160	126320
12927	1	0	0	0	0	0	0	<10	640	49110	83160	144760
12926	2	0	0	0	0	0	0	<10	1580	78380	163240	400910*
12932	2	0	0	0	0	0	0	<10	420	15400	38500	52360
12931	1	0	0	0	0	0	0	<10	320	40020	70840	116390
12934	1	0	0	0	0	0	0	<10	760	23100	93480	130900
12933	2	0	0	0	0	0	0	<10	800	43120	81080	158420
12912	CONTROL	0	0	0	0	0	0	<10	780	23100	70500	58520
12913	CONTROL	0	0	0	0	0	0	<10	520	24090	51090	167860
12915	CONTROL	0	0	0	0	0	0	<10	760	33880	93410	101640

Treatment*

MONKEY		12	13	14	15	16	17	18	19	20	21	22
12921	2	411020*										
12920	1	400990*										
12923	1	139910	284010	111400	57290	3110	<10	<10	<10	0	0	0
12922	2	429000*										
12927	1	610990*										
12926	2											
12932	2	80900	69460	239720	119290	119090	61910	141370*				
12931	1	555680*										
12934	1	376560	199320	287500	440920*							
12933	2	641960*										
12912	CONTROL	429210*										
12913	CONTROL	517440*										
12915	CONTROL	344960	401120*									

* =Treatment

TABLE 5

DETAILED PARASITEMIA OF AOTUS VACCINATED WITH *Plasmodium vivax* DNA VACCINES BASED ON PvCSP, PvSSP2, PvMSP-1p42, PvAMA1, and PvDBP (regions II-IV) alone or in combination

Parasites x cmm

		PI/DAY												
MONKEY	GROUP	1	2	3	4	5	6	7	8	9	10	11	12	13
86016	1	0	<10	<10	<10	180	130	410	810	5010	19890	27110	20960	36820
87057	1	0	<10	<10	<10	260	1410	2120	880	15400	1090	8020	3960	6690
12791	1	<10	<10	<10	<10	1260	2640	6220	10720	24760	7610	3990	9950	2010
88039	2	0	<10	<10	<10	460	195	6890	11620	61600	46070	25520	20990	27110
86068	2	0	<10	<10	<10	140	100	350	<10	>10	320	980	490	<10
12790	2	0	<10	<10	<10	1340	2480	3080	5730	13860	1010	1120t		
88048	3	<10	<10	<10	<10	<10	120	<10	<10	>10	270	460	620	<10
12864	3	0	<10	<10	<10	380	590	1060	2130	18410	8910	12010	10680t	
12793	3	0	<10	<10	<10	710	1750	3490	3640	12330	12320	5810	590	<10
88047	4	0	<10	<10	<10	390	620	1850	1120	27790	13860	9240	8370	7940
12874	4	0	<10	<10	<10	490	1860	3990	1950	9280	26940	13810	5970t	
12792	4	<10	<10	<10	<10	660	1980	7010	12510	29280	33560	16540	7910	1940t
86019	5	0	<10	<10	<10	220	570	1040	1150	4620	1590	8640	1750	1920
12770	5	0	<10	<10	<10	890	6030	6010	15500	19960	8760	4510t		
12795	5	0	<10	<10	>10	520	1530	15510	21970	46200	46200	33040	18090	13560
12802	5	0	<10	<10	>10	610	3020	12940	24500	16940	10780	21010	12390	10110
12807	5	<10	<10	<10	>10	940	5970	13860	22500	86240	35420	27110	44660	29910
12810	DEAD													
12819	5	0	<10	<10	>10	920	1420	3930	8100	30800	8980	1160	560	<10
12676	5	0	<10	<10	>10	810	4960	8990	23840	36970	35420	25500	26180	8940
87024	6	0	<10	<10	<10	620	2110	1750	2010	13860	18090	10110	19770	12060
12787	6	0	<10	<10	<10	610	1020	1880	3740	1780	1500	810t		
12798	6	0	<10	<10	>10	400	2010	2010	6700	40040	21540	27000	13960	24090
12806	6	<10	<10	<10	>10	880	1670	7810	10870	56980	43120	24020	21560	27090
12808	6	0	<10	<10	>10	580	1350	3810	9210	55440	49280	34010	16940	20020
12812	6	0	<10	<10	>10	890	2210	4010	7120	43120	35510	13520	4970	3090
12820	6	0	<10	<10	<10	390	860	3080	1120	24110	21560	10020	11890	2960
11937	6	0	<10	<10	>10	890	1510	7560	15370	18490	3070	7740	5860	8920
88002	7	0	<10	<10	<10	980	1830	8240	15750	43120	50820	28510	22100	8970
12789	7	0	<10	<10	<10	740	2620	1970	1220	26740	6110	5890	6990	1100
12799	7	0	<10	<10	>10	1040	2110	14320	22840	73920	45330	39090	36960	18040
12809	7	0	<10	<10	>10	460	1690	3200	7500	78530	59060	38500	35420	42000
12811	7	0	<10	<10	>10	640	4860	5860	5620	47760	26180	3910	6910	4280
12814	7	0	<10	<10	>10	560	1980	2590	6500	18090	27320	19910	19840	9010
11928	7	0	<10	<10	>10	1420	4620	10500	16750	69300	41580	30090	33810	18110
11968	7	0	<10	<10	>10	1060	1720	3000	10870	21560	18480	13910	7890	19500t
12893	CONTROL	0	<10	<10	<10	370	710	2110	6450	26180	20010	11040	1050	1850
12895	CONTROL	<10	<10	<10	<10	780	1520	1330	5700	27720	27720	12910	30800	18020

t=treated *=Transfusion

TABLE 5 cont..

DETAILED PARASITEMIA OF AOTUS VACCINATED WITH Plasmodium vivax DNA VACCINES BASED ON PvCSP, PvSSP2, PvMSP-1p42, PvAMA1, and PvDBP (regions II-IV) alone or in combination

Parasites x cmm

		PI/DAY												
MONKEY	GROUP	14	15	16	17	18	19	20	21	22	23	24	25	26
86016	1	22590	13500	9290	3210	1210	660	<10	<10	0	0	0	0	0
87057	1	<10t												
12791	1	<10t												
88039	2	24390	18020	12320	4880	5110	3940	345	49	960	260	48	390	<10
86068	2	<10t				DIED								
12790	2													
88048	3	<10	<10	<10	<10	<10	<10	<10	0	0	<10	0	0	0
12864	3													
12793	3	<10	<10	0	0	0	0	0	0	0	0	0	0	0
88047	4	1780	2920	3080	660	<10	<10	<10	<10	<10	0	0	0	0
12874	4													
12792	4													
86019	5	<10	<10	<10	<10	0	0	0	0	0	0	0	0	0
12770	5													
12795	5	12500	2490	580	<10	<10	0	0	0	0	0	0	0	0
12802	5	1870	6910	1330	12890	5820	9260	330	460	<10	<10	<10	0	0
12807	5	16500	10500	12110	4420	3770	1720	1030	<10	<10	<10	<10	<10	<10
12810	DEAD													
12819	5	<10	<10	<10	0	0	0	0	0	0	0	0	0	0
12676	5	8010	610	<10	<10	0	0	0	0	0	0	0	0	0
87024	6	9970	1880	<10	<10	0	0	0	0	0	0	0	0	0
12787	6													
12798	6	2360	4660	3990	4810	1040	890	340	820	<10	<10	<10	0	0
12806	6	18090	3950	2950	3770	1010	<10	<10	<10	<10	<10	<10	<10	<10
12808	6	13590	11090	2050	1290	<10	0	<10	<10	<10	<10	<10	<10	0
12812	6	790t												
12820	6	10590	6890	2020	940	<10	0	<10	0	0	0	0	0	0
11937	6	5110	4010	7590	5620	2960	2990	2950	3180	1560	4660	4020	10090	4180
88002	7	3890	810	<10	<10	<10	0	0	0	0	0	0	0	0
12789	7	<10	<10	<10	<10t	0	0	0	0t					
12799	7	8910	7710	9020	1340	870	<10	<10	<10	<10	<10	<10	0	0
12809	7	21560	27410	10550	2220	4020	1390	163	380	1240	740	760	980	390
12811	7	880	1090	<10	<10	0	0	0	0	0	0	0	0	0
12814	7	2930	3560	1040	1170	<10	<10	<10	0	<10	<10	<10	0	0
11928	7	15920	8990	13500	3960	1890	980	380	<10	<10	<10	<10	<10	<10
11968	7										0	0	0	0
12893	CONTROL	<10	<10	<10	<10	0	0	0	0	0t				
12895	CONTROL	7930	870	1460	1910	590	0	<10	0t					

t=treated *=Transfusion

TABLE 5 cont..

DETAILED PARASITEMIA OF AOTUS VACCINATED WITH Plasmodium vivax DNA VACCINES BASED ON PvCSP, PvSSP2, PvMSP-1p42, PvAMA1, and PvDBP (regions II-IV) alone or in combination

Parasites x cmm

			PI/DAY		
MONKEY	GROUP		27	28	29
86016		1	0	0	0
87057		1			
12791		1			
88039		2	0	0	0
86068		2			
12790		2			
88048		3	0	0	0
12864		3			
12793		3	0	0	0
88047		4	0	0	0
12874		4			
12792		4			
86019		5	0	0	0
12770		5			
12795		5	0	0	0
12802		5	0	0	0
12807		5	<10	0	0
12810	DEAD				
12819		5	0	0	0
12676		5	0	0	0
87024		6	0	0	0
12787		6			
12798		6	0	0	0
12806		6	0	0	0
12808		6	0	0	0
12812		6			
12820		6	0	0	0
11937		6	2110t		
88002		7	0	0	0
12789		7			
12799		7	0	0	0
12809		7	290	<10	0
12811		7	0	0	0
12814		7	0	0	0
11928		7	0	0	0
11968		7	0	0	0
12893	CONTROL				
12895	CONTROL				

TABLE 6

DETAILED PARASITEMIA OF HETEROLOGOUS *Plasmodium falciparum* CAMP STRAIN
BLOOD STAGE CHALLENGE OF HYPERIMMUNE AOTUS MONKEYS

Parasites x cmm
DAY/PI

MONKEY	1	2	3	4	5	6	7	8	9	10	11	12	13
12749	0	0	0	0	0	0	0	0	0	0	0	0	0
12759	0	0	0	0	0	0	0	<10	590	870	1440	720	1220
12739	0	0	0	0	0	0	0	0	0	0	0	0	0
12756	0	0	0	0	0	0	0	0	<10	380	140	170	<10
12757	0	0	0	0	0	0	<10	<10	2980	960	1540	770	910
12765	0	0	0	0	0	0	0	<10	<10	1010	1100	1610	790
12763	0	0	0	0	0	0	<10	<10	670	1260	2300	2540	1250
12730	0	0	0	0	0	0	0	0	0	0	0	0	0
12910 control	0	0	0	0	0	<10	>10	1480	43120	53490	290250	484500*	
12911 control	0	0	0	0	0	<10	<10	>10	31600	42920	264750	519000*	
12943 control	0	0	0	0	0	0	<10	<10	12010	21560	66750	176250	767250*

MONKEY	14	15	16	17	18	19	20	21	22	23	24	25	26
12749	0	0	0	0	0	0	0	0	0	0	0	0	0
12759	2520	1340	560	<10	0	0	0	0	0	0	0	0	0
12739	0	0	0	0	0	0	0	0	0	0	0	0	0
12756	<10	<10	<10	<10	0	0	0	0	0	0	0	0	0
12757	<10	<10	<10	0	0	0	0	0	0	0	0	0	0
12765	9890	6710	750	<10	<10	0	0	0	0	0	0	0	0
12763	10920	5820	180	<10	<10	0	0	0	0	0	0	0	0
12730	>10	<10	<10	120	<10	0	0	0	0	0	0	0	0
12910 control													
12911 control													
12943 control													

* Treatment

TABLE 7

DETAILED PARASITEMIA OF AOTUS VACCINATED WITH A PLASMID DNA VACCINE EBA-175, AMA-1, MSP-1 AS A COMBINATION AND RE-CHALLENGED WITH A *P. falciparum* FVO STRAIN

Parasites x cmm

	DAY/PI											
MONKEY	6	7	8	9	10	11	12	13	14	15	16	17
GROUP 4												
12863	0	0	0	0	0	<10	<10	>10	9090	15960	159020	197120
12865	0	0	0	0	0	<10	<10	>10	1620	13040	21100	72380
12866	0	0	0	0	0	<10	<10	<10	12110	31500	98060	324060
12869	0	0	0	0	0	<10	<10	<10	12990	12190	94510	56840
12870	0	0	0	0	<10	<10	<10	<10	15240	9560	84770	266010
12872	0	0	0	0	0	<10	<10	<10	1640	1980	65510	9010
12873	0	0	0	0	0	<10	<10	>10	181690	80060	663140t*	309540
12875	0	0	0	0	0	<10	<10	<10	1330	21000	126120	100100
GROUP 5												
12879	0	0	0	0	0	<10	<10	<10	5090	12160	50820	93940
12822	0	0	0	0	0	<10	<10	>10	480	9910	18010	51980
12823	0	0	0	0	0	<10	<10	>10	1310	4890	27000	9180
12829	0	0	0	0	0	<10	<10	>10	1540	18110	76090	78860
12832	0	0	0	0	0	<10	<10	<10	2050	16500	79260	15410
CONTROL												
12903	<10	<10	>10	660	640	77010	198030	246400	548910t			
12904	0	0	0	0	0	<10	<10	<10	2030	750	3090	2110

MONKEY	18	19	20	21	22
GROUP 4					
12863	306000	420910t*	216140m**		
12865	75590	379910	144090	116150	42750m**
12866	310900	528000t*	276320m**		
12869	86240	96040	47090	7440	1010m**
12870	369600	410050t*	114040m**		
12872	76560	91500	36960	74250m**	
12873	276000	533990m*	DIED		
12875	234080	422090t*	190200m**		
GROUP 5					
12879	92400	61500	15460	9360	1840m**
12822	344960	400110t*	297000m**		
12823	190810	63090t*	13960m**		
12829	149930	153000	120010	93620	DIEDm**
12832	278010	296010	324000	333710m*	DIED
CONTROL					
12903					
12904	1720	3010	980	1290	<10m**

*=Treatment **=Mefloquine